

STUDIES ON THE PREPARATION AND METHYLATION OF
ALKALI METAL DERIVATIVES OF SUCROSE

Thesis submitted for the degree of

MASTER OF SCIENCE

by

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The studies described in this thesis are part of a larger project carried out at the Arthur D. Little Research Institute, Inveresk, Midlothian. The original aim of the project was defined as "An Exploration of the Usefulness of the Sodium Sucrates in Condensation with Chloro Compounds". The author's part in this work comprised an investigation into various methods of preparing sodium sucates, i.e. true sodium salts of sucrose, $C_{12}H_{22-x}O_{11}Na_x$, and a subsequent study of the structure of these intermediate sucates by quantitative replacement of sodium atoms by methyl groups. Determination of the location of the methyl groups in the resulting methyl sucroses would indicate the positions of the sodium atoms in the original sucates.

It was considered that these methylation studies would give valuable information on the reactivity and course of reaction of sodium sucates in condensations with organic chlorine compounds, which formed the basis of the larger project.

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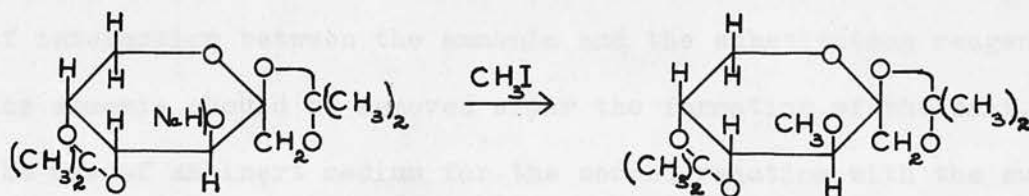
INTRODUCTION

The addition compounds of sucrose with metallic hydroxides, sometimes called saccharates, are well known. Percival¹ formed a potassium hydroxide addition compound of sucrose with the approximate formula $C_{12}H_{22}O_{11} \cdot 3KOH$ by treatment with strong alcoholic potassium hydroxide. The compound was methylated with dimethyl sulphate to give a sucrose methyl ether which was proved to be 1',6,6'-tri-O-methylsucrose, indicating that the potassium hydroxide was associated with the primary alcohol groups in the original addition compound. Much less is known, however, of the alkali metal sucates, i.e. the metallic salts of sucrose or true substitution products:

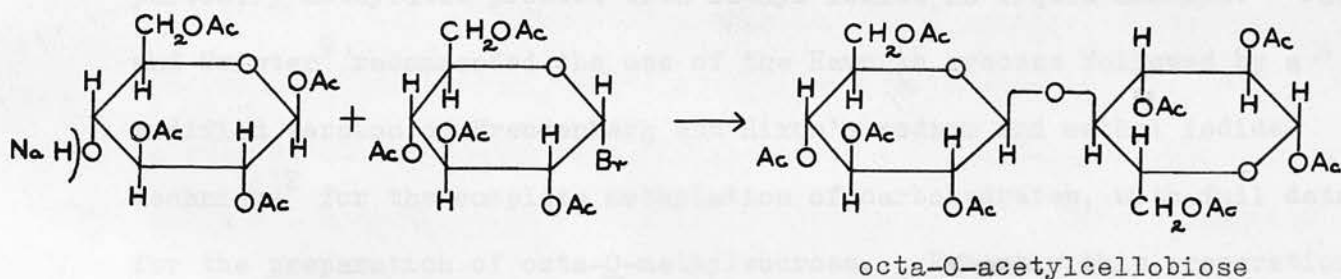


These were the primary derivatives under investigation as intermediates for further condensation reactions.

The Williamson ether synthesis can be applied to alkali metal substituted derivatives of carbohydrates but is limited by the low solubility of most sugars in solvents inert to sodium, unless they are initially highly substituted. Freudenberg and Hixon² first used this method to react di-O-isopropylidenehexoses in ether or benzene solution with sodium and the resultant salts were methylated to give the appropriate methyl ethers. Thus 1,2:4,5-di-O-isopropylidene-D-fructose in benzene formed the 3-sodio salt which reacted with methyl iodide to give the 3-O-methyl ether.



Gilbert, Smith and Stacey³ avoided the use of solvents completely in their syntheses of cellobiose and gentiobiose. Thus extremely pure 1,2,3,6-tetra-O-acetyl- β -D-glucose was fused with one equivalent of sodium to give the 4-sodio derivative. While still in the molten state, this was condensed with acetobromoglucose to form octa-O-acetylcellobiose. Octa-O-acetylgentiobiose was prepared in the same way from 1,2,3,4-tetra-O-acetyl- β -D-glucose and acetobromoglucose.



One solvent which possesses the dual properties of solubility for carbohydrates and inertness to sodium is liquid ammonia. Following initial work by Schmid and Becker⁴ and Kraus and White⁵, Muskat advocated its use for the general preparation of alkali metal substituted intermediates from carbohydrates. In his first paper⁶ he described its excellent solvent properties for all the ordinary sugars, their methylated, acetylated and acetonated derivatives, and he also found that the alkali metals dissolved readily to form salts with the evolution of hydrogen. The potassium salts particularly were shown to be reactive intermediates for the introduction of alkyl, aryl and acyl groups into the molecule. In his second paper⁷ he described more fully his experimental procedure

and apparatus. In addition he recommended that whenever the possibility of interaction between the ammonia and the substituting reagent arose, the ammonia should be removed after the formation of the salt, prior to the use of an inert medium for the second reaction with the substituting reagent.

Muskat's liquid ammonia method has been extensively employed for the methylation of carbohydrates, although chiefly to effect complete methylation in combination with the Haworth dimethyl sulphate-alkali procedure. Hendricks and Rundle⁸ described the preparation of the tetra-O-methyl ethers of glucose, mannose and galactose by first methylating with dimethyl sulphate and then reacting the sodium derivative of the partially methylated product with methyl iodide in liquid ammonia. Pacsu and Trister⁹ recommended the use of the Haworth process followed by a modified version of Freudenberg and Hixon's sodium and methyl iodide technique² for the complete methylation of carbohydrates, with full details for the preparation of octa-O-methylsucrose. Recently this preparation was carried out by Bredereck, Hagelloch and Hambsch.¹⁰

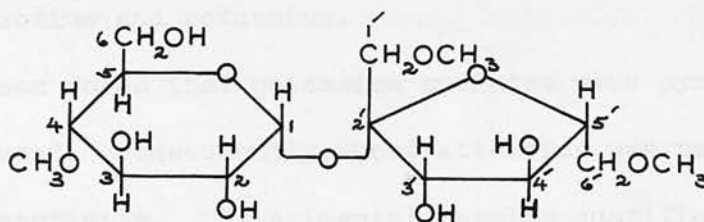
In the case of polysaccharides, where solubility of the starting material is usually not feasible, it has been found possible nevertheless to apply Muskat's method directly for methylation. Polysaccharides such as cellulose¹¹⁻¹³, starch¹⁴⁻²⁰, the Schardinger dextrans²¹ and dextrans^{22,23} have all been successfully methylated by treatment of their sodium or potassium alcoholates with methyl iodide in liquid ammonia. This method has been extensively used both by Freudenberg and coworkers and by Stacey and coworkers.

Few references occur to the preparation of sucrose ethers or esters

by the Muskat method, although Muskat⁶ himself did report the preparation of octa-O-methylsucrose without giving details. Amagasa and Onikura²⁴, however, showed that the method could be readily applied to the preparation of polypotassium derivatives of sucrose. High potassium content compounds were mixtures of hexa- and heptapotassium sucates, almost insoluble in liquid ammonia and instantly pyrophoric in air. Amagasa and Hori²⁵ found that these were reactive towards benzyl chloride, but only in the absence of ammonia as Muskat had suggested. Benzyl chloride reacted directly with polypotassium sucate at 130-140° with the formation of potassium chloride, an oily mixture of hexa- and hepta-O-benzylsucrose and traces of dibenzyl- and tribenzylamine. These benzyl compounds possessed a bitter taste and were readily soluble in ether, acetone, chloroform, benzene and hot alcohol, but almost insoluble in water. They were fairly stable to acid and alkali. Further benzylation formed octa-O-benzylsucrose and acetylation gave a benzylacetylsucrose. Direct reaction of acetyl chloride on the polypotassium sucate was too violent but proceeded smoothly in benzene solution at room temperature with the formation of octa-O-acetylsucrose in reasonably high yield.

Few methylated derivatives of sucrose are known. Fully methylated octa-O-methylsucrose was first prepared by Purdie and Irvine²⁶ and subsequently by various workers.^{28,30,9} Bredereck et al.¹⁰ methylated sucrose with dimethyl sulphate and 30% sodium hydroxide at 60° at pH 10-11, and the partially methylated derivative was converted to the sodium salt before methylation was completed by treatment with methyl iodide or dimethyl sulphate. Kuhn, Trischmann and Löw³¹ succeeded in preparing octa-O-methylsucrose with a single methyl iodide-silver oxide treatment by first dissolving the sucrose in dimethylformamide. It was not obtained crystalline.

Hepta-O-methylsucrose was obtained as a syrup by Haworth.^{27,29} 1',4,6'-Tri-O-methylsucrose was prepared as a syrup by McKeown, Serenius and Hayward.³² Sucrose was treated with triphenylmethyl chloride in pyridine and then with acetic anhydride to give a crystalline tri-O-trityl-penta-O-acetylsucrose, and detritylation of this product by graded hydrolysis with acetic acid, followed by methylation and deacetylation, yielded 1',4,6'-tri-O-methylsucrose. The structure was established by periodate oxidation and by hydrolysis to the corresponding methyl ethers of glucose and fructose.



1',4,6'-Tri-O-methylsucrose

It was concluded that acetyl migration from C₄ to C₆ in the glucose moiety occurred during the methylation reaction. Lemieux and Barrette,³³ however, claim that the penta-O-acetylsucrose was in fact the 2,3,3',4',6-derivative and that acetyl migration did not occur during methylation.

2,3,3',4,4'-Penta-O-methylsucrose has been prepared as a syrup by McKeown and Hayward.³⁴ Tri-O-tritylsucrose was methylated and the trityl groups removed by graded hydrolysis with acetic acid. The structure was proved by hydrolysis to 2,3,4-tri-O-methyl-D-glucose and 3,4-di-O-methyl-D-fructose.

PART I. PREPARATION OF ALKALI METAL DERIVATIVES OF SUCROSE

(a) DISCUSSION

The Alkali Metals

Although the original title of the project specifies sodium, the properties of all three of the more common alkali metals, i.e. lithium, sodium and potassium, were reviewed. The solubilities of these three metals in liquid ammonia at -33° are 10.9, 24.6 and 49.0 g./100 g. ammonia respectively.³⁵ Lithium, however, was found to be unsuitable compared with sodium and potassium.

It has been noted that potassium sucates were pyrophoric when highly substituted; consequently, most attention was paid to sodium with its moderate reactivity. Experimental results justified these conclusions.

Use of Solvents other than Liquid Ammonia

While the properties of liquid ammonia already outlined made it an obvious medium for the preparation of sucates, it was thought that more conventional solvents could be found in which sucrose was appreciably soluble and in which alkali metals would react preferentially with the sucrose molecule. The use of such solvents would obviate the need for such special apparatus and techniques as liquid ammonia demanded. In addition, as the presence of ammonia has been stated^{7,25} to interfere seriously with subsequent coupling reactions, it was hoped to find a suitable medium in which eventually both preparation and condensation of sucates could be carried out, thus eliminating isolation of the intermediates.

Reference is made to the literature review of Kononenko and Herstein³⁶

on non-aqueous solvents for sucrose which are of sufficient stability to be considered as reaction media. Six of the solvents listed show reasonably high solubility for sucrose, viz., morpholine, dimethylsulphoxide, N-methyl-2-pyrrolidone, dimethylformamide, pyridine and 2-methylpiperazine: preliminary experiments showed that, of these, only pyridine and morpholine were sufficiently stable to sodium to be considered as media in the sucrose-sodium reaction.

Preparation of Sucrose Derivatives in Pyridine

Pyridine was the first conventional solvent to be tried possessing the advantages of solubility for sucrose, high stability and purity. While it is known that pyridine does react with sodium to form a "disolvate", $\text{Na} \cdot 2\text{C}_5\text{H}_5\text{N}$,³⁷ it was thought that this reaction would take second place to that between sodium and sucrose. When sucrose dissolved in pyridine is treated with sodium, no hydrogen is evolved. On heating, the metal darkens in colour, liquefies above 100° , and a sodium derivative is rapidly deposited as a white crystalline derivative.

A series of experiments was conducted to form sodium-sucrose derivatives in pyridine and the results are shown in Table 1.

In expt 1, where one atom of sodium was reacted with a solution of sucrose in pyridine at 110° under nitrogen, a white crystalline precipitate A was isolated which contained 2.9 sodium atoms per sucrose molecule. A product B was obtained from the filtrate by ether precipitation; analysis showed it was mostly sucrose. 2.33 Atoms of sodium were introduced initially in expt 2, where the main product was a trisodium derivative, with a negligible solvent content.

In expts 3, 4 and 5, attempts to make tetra-, hexa- and octasodium derivatives were successful on a sodium/sucrose basis, with increasing

Table 1

Sodium Derivatives of Sucrose prepared in Pyridine and Morpholine

Expt No.	Atoms Na added/ sucrose mol.	Reaction products	Na %	Sucrose (as $C_{12}H_{22}O_{11}$) %	N %	Na recovery %	Sucrose recovery %	Na atoms/ sucrose mol.	$[\alpha]_D^{20}$ (c, 1; water)
1	1.00	A	13.64	69.0	0.84	89.5	30.4	2.93	+42.4°
		B	0.00	87.2	N.D.	-	59.1	-	58.0
2	2.33	A	14.41	70.4	0.00	94.4	72.2	3.05	44.8
		B	0.22	61.7	N.D.	0.4	19.4	-	43.9
3	4.00	A	17.96	64.9	0.10	95.9	93.2	4.13	41.5
4	6.00	A	23.18	56.8	0.26	94.4	93.4	6.06	35.3
5	8.00	A	28.30	50.5	0.89	97.7	93.8	8.33	31.3
6	4.00	A	18.40	60.7	N.D.	85.7	76.1	4.51	38.9
		B	17.14	43.7	N.D.	10.8	7.3	5.84	N.D.
7	4.00	A	15.71	54.6	12.6*	93.3	87.3	4.28	35.9
8	1.00	A	7.12	74.0	12.9*	91.4	63.1	1.43	48.8
		B	0.05	48.2	N.D.	0.3	23.9	-	N.D.
9	0.70	A	5.31	73.4	15.6*	86.2	56.8	1.08	46.9
		B	0.09	55.2	37.1*	0.6	28.1	-	36.4
10	1.00	A	17.40	48.6	14.0*	90.2	17.0	5.33	30.5
		B	0.14	56.4	39.8*	2.1	56.2	-	37.6

N.D. - not determined

* Nitrogen figures recalculated as % solvent

solvent content. No appreciable amount of B was precipitated.

The main products A did not analyse satisfactorily as true sodium-substituted derivatives of sucrose, but were somewhat better as sodium hydroxide addition compounds, $C_{12}H_{22}O_{11} \cdot xNaOH$. Recalculating on this basis, the sum of $C_{12}H_{22}O_{11}$ and NaOH contents are 92.7, 95.5, 96.2, 97.1 and 99.7% in expts 1-5 respectively. On the assumption that the barium oxide-dried pyridine was not quite moisture-free, a fresh sample was given extensive drying before another tetrasodium derivative was prepared in it (expt 6). The reaction was sluggish, requiring four hours for complete sodium removal compared to 45 minutes previously, with a corresponding drop in sucrose recovery, indicating decomposition during the reaction.

Preparation of Sucrose Derivatives in Morpholine

Morpholine was dried over barium oxide and fractionally distilled. Sucrose in solution reacted with sodium in a manner similar to that in pyridine.

In expt 7, four atoms of sodium were introduced into a sucrose solution in morpholine under nitrogen. After 45 minutes at 110° a heavy yellow precipitate A was isolated, but again it did not analyse completely as a true sodium salt $C_{12}H_{18}O_{11}Na_4$.

Expts 8 and 9 were attempts to form the monosodium derivative using smaller proportions of sodium (1.0 and 0.7 atoms/sucrose molecule respectively) and this was achieved on a sodium-sucrose basis in expt 9. The reactions were carried out as in expt 7, followed by ether precipitation of a syrup B from the supernatant liquid of the white precipitate obtained in each case. Once more the sodium + sucrose + morpholine contents failed to add up to 100%.

For expt 10, morpholine was dried over sodium and redistilled before use. An attempt to repeat the preparation of a monosubstituted derivative under more rigidly anhydrous conditions gave a totally different product still analysing unsatisfactorily. The reaction was prolonged, with corresponding loss of sucrose.

Summary of Work with Conventional Solvents

In all cases the solvents examined entered into some reaction with sodium. Even where this seemed slight, the resulting sodium-sucrose derivatives analysed more closely to sodium hydroxide addition compounds, contaminated with solvent residue. In view of the unsatisfactory analyses no further work was carried out on conventional solvents; attention was directed to the preparation of sodium sucates in liquid ammonia.

Preparation of Sucates in Liquid Ammonia

Reference has already been made to the reaction between sucrose dissolved in liquid ammonia and potassium.²⁴ When the alkali metal is dissolved in liquid ammonia an intense deep blue colour is formed, but this is discharged as the metal reacts with the carbohydrate molecule, with evolution of hydrogen.

The procedure used to prepare sodium sucates in this manner is described in the Experimental section and the apparatus is illustrated in Figure 1.

The monosodium sucate prepared in expt 1 (Table 2) was isolated as a white, crystalline mass. Analysis and, particularly, its high sucrose content (90.1%) showed clearly that it was a true substitution compound, containing one molecule of ammonia, and not a sodium hydroxide addition compound. ($C_{12}H_{22}O_{11}NaOH.NH_3$ requires Na, 5.76; $C_{12}H_{22}O_{11}$, 85.72; NH_3 , 4.27. $C_{12}H_{21}O_{11}Na.NH_3$ requires Na, 6.03; $C_{12}H_{22}O_{11}$, 89.78; NH_3 , 4.47%).

Table 2

Sodium Derivatives of Sucrose prepared in Liquid Ammonia

Expt No.	Atoms Na added/ sucrose mol.	Reaction time (hr.)	Reaction products	Na %	Sucrose (as C ₁₂ H ₂₂ O ₁₁) %	NH ₃ %	Na recovery %	Sucrose recovery %	Na atoms/ sucrose mol.	$[\alpha]_D$ (c,l; water)
1	1.00	0.5	Sucrate A	5.93	90.1	4.64	96.2	98.2	0.98	+56.6°
2	1.00	0.5	Sucrate K	9.67*	88.9	2.76	94.3*	99.1	0.95*	55.5
3	1.00	0.5	Sucrate C	7.01	89.7	3.46	90.8	78.1	1.16	56.1
4	2.00	0.8	Sucrate D	1.15	93.2	5.09	3.5	18.9	0.18	59.4
			Not isolated							
5	4.00	4.0	Sucrate A	19.89	77.1	1.19	92.2	96.1	3.84	46.4
6	6.00	>12	Sucrate A	23.47	69.9	2.02	73.8	88.7	5.00	41.4

* These figures refer to potassium

In expt 1, the product started precipitating from solution before half an atom of sodium was added but when twice the volume of ammonia was used the product still failed to stay in solution. It has been reported by Amagasa and Onikura²⁴ that monopotassium sucrate is soluble in liquid ammonia at room temperature under pressure; when prepared at -33° at atmospheric pressure (expt 2), this compound was by comparison no more soluble than the sodium salt, was less crystalline and contained only 0.624 mol. ammonia. Muskat⁷ has reported that lithium salts are more soluble than potassium or sodium salts and it was found that 2.5 atoms of lithium could be added to 5 g. sucrose in 50 ml. liquid ammonia before precipitation started; after this, however, the reaction slowed down, requiring $2\frac{1}{2}$ hours to form a sucrate containing 3.73 atoms lithium/sucrose molecule.

At first monosodium sucrate A (expt 1) appeared to be a homogeneous, crystalline compound. Expt 3, in which a monosodium sucrate was filtered on a sintered glass crucible attached to the reaction flask, showed that this was not so. The insoluble fraction C retained by the filter was a mixture of mono- and higher substituted sodium sucates, while fraction D, containing chiefly sucrose and ammonia, was isolated by evaporation of the filtrate. When similar attempts were made to fractionate the di- and tetrasodium derivatives, their fine state of division made filtration impossible.

A tetrasodium derivative (expt 5) was isolated as a white, hygroscopic, non-pyrophoric powder containing a much reduced quantity of ammonia. The sum of the sodium, sucrose and ammonia contents amounts to 98.2%, indicating that it is mostly a true sucrate, although it is progressively more difficult, with the higher substituted and correspondingly more hygroscopic

compounds, to exclude moisture completely during isolation and analysis.

($C_{12}H_{18}O_{11}Na_4$ requires Na, 21.38; $C_{12}H_{22}O_{11}$, 79.5%). When the preparation of a hexa-sodium derivative was attempted (expt 6), a reaction time of more than 12 hours was required, while the derivative isolated actually proved to be only penta-substituted due to some of the sodium being deposited in the necks of the reaction vessel and consequently lost to the reaction.

In an attempt to reduce the reaction times, sodium was added as a solution in liquid ammonia rather than in the form of small pieces, but the reaction time was not decreased. For the same purpose Scherer et al.³⁸ claimed that the addition of 1% of sodium halide to liquid ammonia had a catalytic effect, reducing the time of formation of trisodium cellulosate from 90 to 10-12 minutes. It was found, however, that the reaction time for a tetrasodium sucrate was $6\frac{1}{2}$ hours in the presence of 0.5 g. sodium chloride, compared with 4 hours in its absence.

Preparation of higher substituted derivatives has not been attempted by this metal-ammonia technique because of the prolonged reaction times. Higher substituted sodium sucates are more conveniently prepared by the use of sodamide.³⁹

(b) EXPERIMENTAL

The sucrose used in these investigations was B.D.H. 'Analar' grade without further purification.

Sodium was determined in samples by ashing and conversion to sodium sulphate, and sucrose was determined by the Somogyi method described below.

The solvent content of the prepared derivatives was calculated directly

from the nitrogen content which was determined by the micro-Kjeldahl method of Cole and Parks⁴⁰, except for pyridine-nitrogen which gave unsatisfactory results; in this case results were obtained by the micro-Dumas method.⁴¹

Estimation of Sucrose by the Somogyi Micro-copper Method

The method was first standardised using a known weight of sucrose (500 mg.) which was hydrolysed with 0.2N sulphuric acid (50 ml.) at 100° for 60 minutes; the solution was then neutralised with 2N sodium hydroxide (phenol red indicator) before being made up to 100 ml. Aliquots of this solution were used to prepare standard solutions of various dilutions. Portions (5 ml.) of each of these mixed with Somogyi copper reagent⁴² (5 ml.) were heated in a boiling water-bath for 15 minutes, cooled to 30° and, after the addition of 2.5% potassium iodide solution (2 ml.) and N sulphuric acid (5 ml.), the solution was titrated with 0.005 N sodium thiosulphate. Starch indicator was only introduced near the end-point. The relationship of the reduction equivalent to concentration was linear over the range 0.25-1.5 mg. sucrose.

Found: 1 mg. sucrose (after hydrolysis) \equiv 6.61 ml. 0.005 N $\text{Na}_2\text{S}_2\text{O}_3$

When this method was applied directly to sodium derivatives of sucrose, the presence of residual solvent interfered and this was removed using a cation-exchange resin. The derivative (500-700 mg.) was dissolved in water (10 ml.) and the solution was passed through an Amberlite resin IR-120- H^+ column (10 ml.) with subsequent washing with water (100 ml.). The effluent and washings were concentrated in vacuo to a syrup before the reduction equivalent was determined as above: thus, when sucrose (500 mg.) and pyridine (102 mg.) were dissolved in water (10 ml.), containing 2N sodium hydroxide (2 ml.), and treated in this manner, a 99.3% recovery of

sucrose was estimated.

Preparation of Sucrose Derivatives in Pyridine

The apparatus used for these preparations consisted of a three-necked, 250 ml., round-bottomed flask as the reaction vessel, fitted with condenser, stirrer and side-arm for introduction of the reactants and oxygen-free nitrogen.

Initially, pyridine ('Analar' grade) was dried over barium oxide and redistilled; for expt 6, however, it was dried over caustic potash and redistilled six times over phosphorus pentoxide to ensure complete absence of moisture. Sucrose (5 g.) was dissolved in pyridine (100 ml.) before the addition of the required amount of freshly-cut sodium. The mixture was rapidly stirred at 110° - 115° for 45 minutes under nitrogen and was allowed to stand overnight at room temperature. The resultant precipitate A was filtered, washed with pyridine and dry, alcohol-free ether and dried in vacuo to a white, crystalline powder. Unreacted sucrose was recovered by treating the filtrate with excess ether (400 ml.) to effect complete precipitation; product B was centrifuged, washed with ether and dried to a yellow powder.

Preparation of Sucrose Derivatives in Morpholine

Experiments were conducted exactly as for pyridine. For expts 7-9 morpholine was dried over caustic soda and redistilled; in expt 10 morpholine was dried over sodium and redistilled.

Preparation of Sucrates in Liquid Ammonia

The apparatus (Figure 1) consisted of a 250 ml., three-necked, round-bottomed flask A with a condenser B filled with solid carbon dioxide-methanol cooling mixture fitted to one side-neck. For the monosodium sucrate magnetic stirring was sufficient, leaving the centre-neck stoppered;

sucrose was estimated.

Preparation of Sucrose Derivatives in Pyridine

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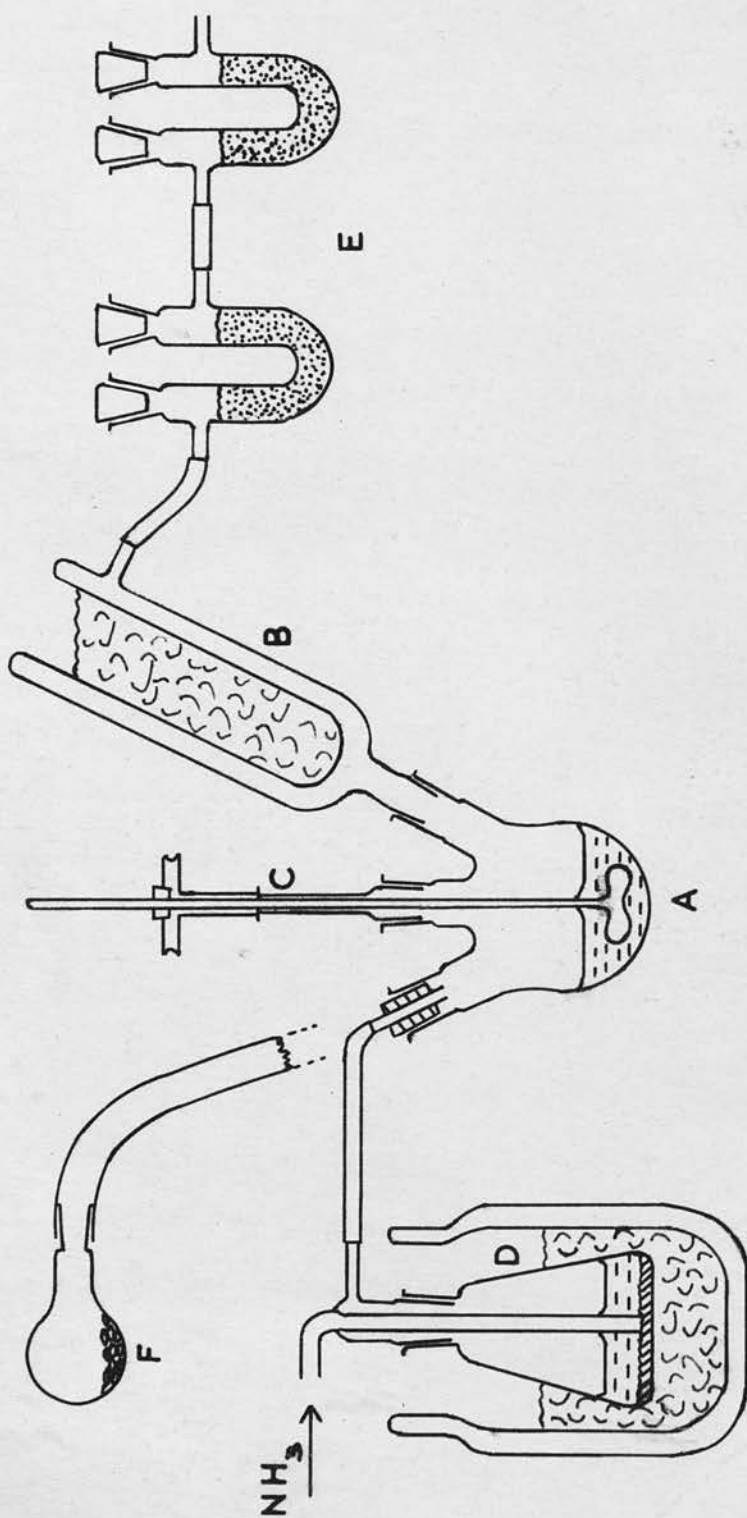
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Preparation of Sucrates in Liquid Ammonia

The apparatus (Figure 1) consisted of a 250 ml., three-necked, round-bottomed flask A with a condenser B filled with solid carbon dioxide-methanol cooling mixture fitted to one side-neck. For the monosodium sucrate magnetic stirring was sufficient, leaving the centre-neck stoppered;

FIGURE I



for higher sucrates a more powerful 'trubore' stirrer C, with its precision joint lubricated with silicon grease, was used through the centre-neck. Ammonia and sodium were introduced through the third neck. The ammonia was taken from a gas cylinder through a pressure-reducing unit and was dried by condensing over large pieces of sodium in a separate flask, D, cooled by carbon dioxide-methanol mixture. On reheating to room temperature, the dry ammonia was distilled over into the reaction flask A, which soon became coated with ice. The outlet of the condenser was protected against ingress of water vapour by two barium oxide U-tubes, E. The sodium, precut into small pieces, was contained in a small round-bottomed flask F, connected to flask A by a close-fitting, thick-walled rubber tube; one or more pieces were added by raising the flask carefully and tapping the required number down the tube.

Anhydrous ammonia was condensed with stirring into the main flask A containing sucrose (5 g.) until the volume was about 50 ml. Sodium was then added gradually in the manner described, allowing the characteristic blue colour of the sodium-ammonia solution to disappear between each addition during the first part of the reaction. Until this stage, when approximately one atom of sodium/molecule of sucrose had been introduced, the reaction was vigorous throughout with rapid evolution of hydrogen and heavy precipitation after less than half an atom had been added. With progressively higher substitution the reaction became much less vigorous and the blue colour so prolonged that sodium was added while the mixture was still blue. Following the last addition, the mixture was stirred until the blue colour disappeared; the ammonia was then allowed to evaporate off under strictly anhydrous conditions, the process sometimes being accelerated by flushing out the flask with dry, oxygen-free nitrogen at 70°. The

isolation was completed by removing as much as possible of the remaining ammonia in vacuo over phosphorus pentoxide.

Analyses of the derivatives prepared and their approximate reaction times are recorded in Table 2. The latter values vary with the size of batch and the efficiency of the stirring as would be expected in a heterogeneous reaction; e.g., to prepare a trisodium succrate on a 25 g. scale, a time of 13 hours was required to clear the blue colour.

The monopotassium succrate (expt 2) was prepared in exactly the same manner as the sodium succrates.

PART II. METHYLATION OF SODIUM SUCRATES

(a) DISCUSSION

Introduction

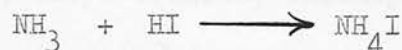
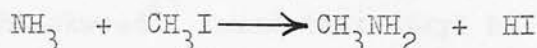
The main aims of methylation have already been expressed in the introductory statement. It was hoped that the methyl ethers of sucrose so prepared would be obtained more or less quantitatively to allow direct correlation of the positions of the methyl groups with those of the sodium atoms replaced.

It was to be expected that any given sodium sucrate would, in fact, be a mixture of sodium sucates of various degrees of substitution and consequently that methylation would produce a similar range of sucrose methyl ethers, which would require some sensitive method of separation before identification. It was hoped initially that each sucrose methyl ether would be homogeneous at its own level of substitution, i.e. it would not be a mixture of isomers; if this were so, sodium sucates would be useful intermediates for the preparation of sucrose methyl ethers. It is now known, however, as described below, that mono-O-methylsucrose prepared from trisodium sucate is a mixture of isomers which are extremely difficult to separate.

Preliminary Methylation Experiments

When methylation of monosodium sucate was first tried in liquid ammonia with methyl iodide, the product was a white solid with a strong 'amine' smell, giving only sucrose on chromatography. As Muskat pointed out,⁷ methylation in the presence of ammonia leads to the formation of methylamines and hydriodic acid which can then decompose the sucate and

also react with ammonia:

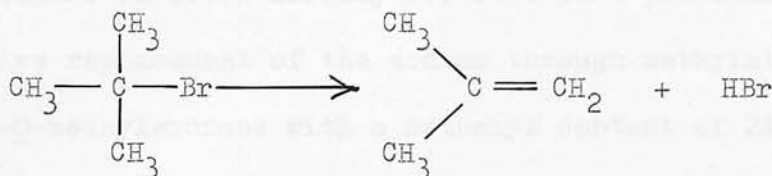


No reaction occurred between monosodium sucrate and methyl iodide while refluxing in acetone, but hydrolysis occurred when reaction was attempted by shaking with methyl iodide in dimethylformamide at room temperature, although the solvent was itself without reaction on the sucrate. Similarly treatment in dimethylsulphoxide at 45° also caused hydrolysis. All attempts to methylate monosodium sucrate, which crystallises with one molecule of ammonia, were unsuccessful. Further preliminary experiments were conducted with tetrasodium sucrate which contains much less ammonia.

Reaction occurred with methyl iodide in dimethylformamide but hydrolysis invariably accompanied methylation. Methyl p-toluenesulphonate, hereafter called methyl tosylate, was introduced as the methylating agent as recommended by Weaver et al.⁷⁶ for cellulose. Apart from this it has not been used before for methylation of carbohydrates. It is a powerful alkylating agent, solid at room temperature (m.p. 28°), and is insoluble in water, so that it can be readily removed after a reaction by water extraction of the products. Methylation and hydrolysis occurred together, however, when it was used in conjunction with dimethylformamide and dimethylsulphoxide.

It was suspected that failure of methyl iodide to react satisfactorily with sodium sucates in dimethylformamide was due to interaction between

the methylating agent and the solvent with the liberation of free acid. Kornblum and Blackwood⁴³ found that alkyl halides in dimethylformamide undergo dehydrohalogenation on standing at room temperature, although methyl iodide reacted less rapidly than secondary or tertiary alkyl halides; from t-butyl bromide, isobutylene was isolated in 46% yield:-



Methyl iodide in the presence of dimethylformamide has been shown to cause hydrolysis. When sucrose was dissolved in dimethylformamide containing methyl iodide at 75°, the specific rotation fell sharply from +74.1° to 41.9° in 1 hour, followed by a gradual increase to 49.2 after 18 hours, when the solution was found to be acid, with sucrose absent. As a result of this finding, dimethylformamide was not used again for sucrate reactions.

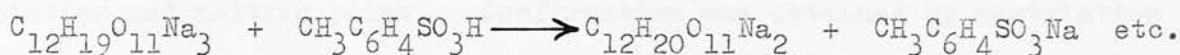
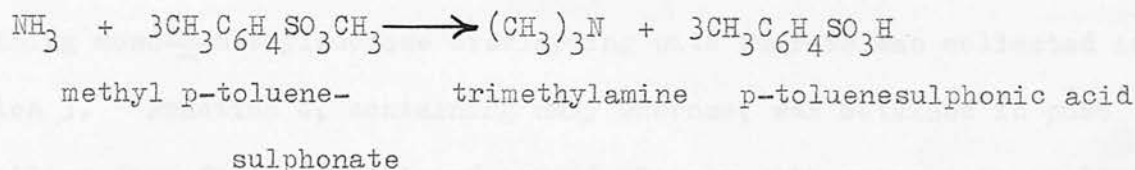
Many other liquids were tried as methylating media. The most successful proved to be tri-O-methylglycerol, which has been suggested⁴⁴ for this purpose. When trisodium sucrate was reacted with the theoretical amount of methyl tosylate in this solvent, a non-reducing glass of 11.7% methoxyl content was obtained containing sucrose and three methylated derivatives.

Methylation of Trisodium Sucrate

It was decided to concentrate attention on methylation of a trisodium derivative, since it was thought that the ammonia content of both mono- and disodium sucates would cause excessive production of sucrose to the detriment of methylation. It was expected that mono-O-methylsucrose

would be formed in sufficient yield to allow its structure and properties to be determined.

When trisodium sucrate was methylated in two batches by reaction with the theoretical weight of methyl tosylate in tri-O-methylglycerol at 110° , the methoxyl contents were found to be 10.5 and 10.6%, consistent with the figure of 11.7% already reported in a previous experiment, whereas quantitative replacement of the sodium through methylation should have given tri-O-methylsucrose with a methoxyl content of 24.2%. This is understandable if it is assumed that the ammonia in the sucrate is first methylated and p-toluenesulphonic acid is formed: this reacts with the sodium sucrate, stripping off the sodium atoms entirely or leaving a sucrate containing fewer atoms than were originally present.



When this hypothesis is applied to 100 g. of trisodium sucrate containing 2.13% ammonia, reaction with methyl tosylate would give, theoretically, 86.0 g. of material with an average methoxyl content of 12.2%. It is shown in the Experimental section that, in fact, 6.082 g. of trisodium sucrate gave 4.962 g. of methylated material (methoxyl content 10.6%), which is equivalent to a yield of 81.6 g. from 100 g. of sucrate.

Methyl tosylate was found to be a satisfactory methylating agent and reacts in the ratio of one molecule to one sodium atom, insomuch as no excess reagent was detected after the reaction and the product was

almost neutral.

Separation of the Products of Methylation

The combined products of two methylation experiments were applied to a loosely-packed cellulose column, which was developed with n-butanol saturated with water. Paper chromatography of samples taken regularly from the eluate showed that only a partial separation resulted. Four fractions were selected arbitrarily, but only sucrose was separated in pure form.

The first fraction was a mixture of di-O-methylsucrose and higher methylated material. Fraction 2 contained some di-O-methylsucrose overlapping chromatographically with the major portion of mono-O-methylsucrose, but without further contamination. The portion of the eluate containing mono-O-methylsucrose overlapping with sucrose was collected in fraction 3. Fraction 4, containing only sucrose, was obtained in pure crystalline form from methanol and agreed closely with sucrose in analysis, rotation and melting point. Confirmation was obtained by acetylation with acetic anhydride and sodium acetate to form crystalline sucrose octa-acetate.

Fraction 2 was refractionated on a smaller, more tightly-packed column to give an almost complete separation of the di-O-methylsucrose and mono-O-methylsucrose components. The latter was refractionated to ensure chromatographic purity. This was checked by paper partition chromatography in other solvents, but no further separation of the mono-O-methylsucrose fraction was obtained at this point. The final product was a non-reducing, pale yellow glass having a methoxyl content and rotation ($[\alpha]_D + 60.6^\circ$ in water) consistent with a monomethyl ether of sucrose. A thorough investigation has been made of this mono-O-methylsucrose.

Characterisation of Mono-O-methylsucrose

Characterisation of the derivative began with periodate oxidation in the dark at 20° and analysis for periodate consumed and formic acid produced. The results showed that mono-O-methylsucrose consumed 2.82 moles of periodate and liberated 0.81 mole of acid in 48 hours, while sucrose in the same time consumed 3.01 moles and liberated 0.65 mole. The sucrose results compare well with those of Fleury and Courtois⁴⁵ who reported a periodate uptake of 3 moles and a formic acid production of 0.70 mole of formic acid after 24 hours. The close comparison between the periodate oxidations of sucrose and mono-O-methylsucrose suggested that substitution had occurred predominantly on the primary alcohol groups, a result which was not confirmed by the hydrolysis experiments.

The mono-O-methylsucrose was hydrolysed at 100° with 0.05N sulphuric acid to give a solution with a negative rotation ($[\alpha]_D - 6.5^\circ$). This eliminated the possibility of the presence of 6-O-methyl-D-fructofuranose as a major component, for this sugar is known⁴⁶ to have a positive rotation. The hydrolysate was neutralised with sodium hydroxide, sodium sulphate precipitated with ethanol, and the centrifugate concentrated to a syrup, paper chromatography of which revealed the presence of a mono-O-methylhexose fraction, besides fructose and glucose.

Cellulose column chromatography was employed to separate the components of the hydrolysate which gave three syrupy fractions, the mono-O-methylhexose fraction A (38.4%), fructose (16.5%) and glucose (30.4%). Fructose was confirmed by formation of crystalline 1,2:4,5-di-O-isopropylidene-D-fructose identical with authentic material. The presence of glucose was confirmed by the isolation of crystalline penta-O-acetyl- β -D-glucose. The combined weights of glucose and fructose were slightly greater than that of the mono-O-methylhexose fraction because of mechanical

loss during the separation.

The methoxyl content of fraction A agreed closely with that of a mono-O-methylhexose and paper chromatography indicated that it contained two glucose and two fructose monomethyl ethers with traces of two others. Separation of the components was effected on thick paper, this being successfully achieved in an ethylmethyl ketone half-saturated with water: ammonia (99:1) solvent system. Six colourless syrups were obtained after charcoal treatment, giving an 86% recovery from fraction A. Chromatographic analysis of the four main components against known glucose and fructose monomethyl ethers enabled a preliminary identification of each to be made, viz.:-

Fraction A1:-	6- <u>O</u> -methyl-D-glucose :	12.5%, 1.0 part
Fraction A2:-	2- <u>O</u> -methyl-D-glucose :	30.4%, 2.4 parts
Fraction A4:-	1- <u>O</u> -methyl-D-fructose :	26.5%, 2.1 parts
Fraction A5:-	3- or 4- <u>O</u> -methyl-D-fructose :	18.4%, 1.5 parts.

For further investigations on the structures of these syrups involving periodate oxidation, the method of Hough et al.⁴⁷ was standardised for the micro-estimation of formaldehyde which is liberated only when the primary hydroxyl groups are unsubstituted.

Identification and Characterisation of Mono-O-methylhexose Components

Syrup A1 agreed closely in methoxyl content and rotation with 6-O-methyl-D-glucose. Periodate oxidation showed a negligible formaldehyde release; 6-O-methyl-D-glucose would be expected to give no formaldehyde production. Partial periodate oxidation by the method of Lemieux and Bauer⁴⁸ formed a formyl ester which, after saponification,

chromatographed identically with that from authentic 6-O-methyl-D-glucose. The structure was finally confirmed by preparation of the phenylosazone which possessed identical properties to those quoted for 6-O-methyl-D-glucosephenylosazone.

Periodate oxidation of fraction A2 followed a similar course to that of 2-O-methyl-D-glucose but lagged behind, e.g. 4.8 moles of periodate were consumed by the authentic material in 48 hours, while A2 consumed 4.5 moles. It may be that the syrup is oxidised at a slightly lower rate than the crystalline sugar. The partial periodate oxidation technique⁴⁸ confirmed its structure as 2-O-methyl-D-glucose. An attempt to form the bright red formazan with triphenyltetrazolium hydroxide⁴⁹ was unsuccessful. Bell and Dedonder⁵⁰ have shown that the formazan is only formed in aldoses with position 2 unsubstituted. The phenylosazone formed from syrup A2 was identified as D-glucosephenylosazone.

The syrupy fraction A4 had a correct methoxyl content for a mono-O-methyl-D-fructose. The results of periodate oxidation show good agreement between A4 and 1-O-methyl-D-fructose, viz. 3.4 and 3.5 moles of periodate consumed and 2.4 and 2.7 moles of formic acid produced in 72 hours respectively, with the syrup again lagging behind the crystalline material. The structure was confirmed by formation of the phenylosazone, which proved to be identical with D-glucosephenylosazone.

The specific rotation ($[\alpha]_D - 54.8^\circ$ in water) of syrup A5 was consistent with that of 3-O-methyl-D-fructose ($[\alpha]_D - 53.5^\circ$)⁵¹ and this was substantiated by the fact that it was obtained crystalline. 4-O-Methyl-D-fructose ($[\alpha]_D - 87.5^\circ$) has not been obtained crystalline⁵² and 6-O-methyl-D-fructofuranose has a positive rotation.⁴⁶ Results of periodate oxidation of the extensively dried material, however, were of a

much lower order than those for 3- and 4-O-methyl-D-fructose. Attempts to form a phenylosazone by the usual method also failed. Finally, the remainder of the syrup was tested by paper ionophoresis against authentic samples of mono-O-methyl-D-fructoses; the major component of the syrup had no appreciable mobility. It has been suggested⁵³ that the anomalous results of periodate oxidation and ionophoresis are due to di-anhydride formation following extensive drying of the syrup. 1,2':2,1'-Di-D-fructose anhydrides are well known⁵⁴ and could conceivably be formed in this case without interference from the methoxyl group on position 3.

Analysis of the mono-O-methylhexose fraction A therefore indicates that it contains 2-O-methyl-D-glucose (2.4 parts), 6-O-methyl-D-glucose (1.0 part), 1-O-methyl-D-fructose (2.1 parts) and most probably 3-O-methyl-D-fructose (1.5 parts). These proportions are, of course, not necessarily those in which substitution originally occurred, since it cannot be assumed that the stripping of sodium atoms caused by the ammonia side-reaction would be uniform over all positions of substitution. Nevertheless, analysis has shown the complexity of the mono-O-methyl-sucrose isolated, thought originally to be non-isomeric. Subsequent extensive paper chromatography succeeded in achieving slight separation of the derivative into two components with trace of a third in benzene: n-butanol:pyridine:water (1:5:3:3).

(b) EXPERIMENTAL

Evaporations were carried out at 45° under reduced pressure. Paper partition chromatography was carried out on Whatman No. 1 paper with the following v/v solvent systems:-

- (A) n-butanol:ethanol:water - 40:11:19
- (B) n-butanol:pyridine:water - 10:3:3
- (C) ethyl methyl ketone half saturated with water:ammonia - 99:1
- (D) benzene:n-butanol:pyridine:water - 1:5:3:3

Sugars were detected by spraying with α -naphthol-phosphoric acid,⁵⁵ benzidine-trichloroacetic acid⁵⁶, p-anisidine hydrochloride⁵⁷ and aniline phthalate.⁵⁸ Aldoses with position 2 unsubstituted were observed using triphenyltetrazolium hydroxide.⁴⁹ R_{Glu} , R_{Fru} , R_{Suc} and R_{F} are the rates of travel relative to glucose, fructose, sucrose and the solvent front respectively. Ionophoresis was carried out in borate buffer (pH 10) at 750 v.⁵⁹ Unless otherwise stated, rotations were measured in water at 20°.

Preliminary Methylation Experiments

Since all these experiments were conducted in a similar manner, the most important are summarised in Table 3. The products of each reaction were tested either for reducing properties (Fehling's Test) or by chromatography in solvents (A) and (B) with benzidine and p-anisidine sprays.

Methyl iodide was dried over sodium and redistilled through a column containing copper turnings; methyl tosylate (methyl p-toluene-sulphonate) was dried in chloroform solution over anhydrous sodium sulphate before distillation, the fraction boiling at 132°/1-2 mm.

Table 3

Preliminary Methylation Experiments

Expt No.	Starting Material	Methylating Agent	Solvent	Reaction Conditions		Product of Reaction
				Time (hr.)	Temp. (°)	
1	Monosodium succrate	Methyl iodide (excess)	Liquid ammonia	0.5	-33	Non-reducing; sucrose only
2	"	Methyl iodide (excess) + Ag_2CO_3	Acetone	4.5	57	No reaction
3	"	Methyl iodide (excess)	Dimethylformamide	24	15	Strongly reducing
4	"	-	Dimethylformamide	24	15	No reaction
5	"	Methyl iodide (excess)	Dimethylsulphoxide	2.5	45	Reaction \rightarrow sucrose only
6	Tetrasodium succrate	Methyl iodide (excess)	Dimethylformamide	20	15	Glucose, fructose and methyl ethers
7	"	Methyl tosylate (theoretical)	Dimethylformamide	2	75	" "
8	"	Methyl tosylate (theoretical)	Dimethylsulphoxide	1.5	100	" "
9	"	Methyl tosylate (excess)	-	16	75	Charring
10	Trisodium succrate	Methyl tosylate (theoretical)	Tri-O-methyl-glycerol	5	110	Non-reducing; sucrose and methyl ethers.

being collected. Both dimethylformamide and dimethylsulphoxide were purified by shaking with anhydrous barium oxide and centrifuging; the centrifugate was distilled, the fractions boiling at $56.5^{\circ}/18$ mm. and $76-9^{\circ}/7$ mm. being collected respectively.

Tri-O-methylglycerol was prepared from epichlorhydrin.⁶⁰ After initial treatment with caustic soda and methanol to form di-O-methylglycerol, methylation was completed using sodium and dimethyl sulphate. It was dried thoroughly by distillation over sodium at $61-2^{\circ}/30$ mm.

In expt 1, Table 3, a monosodium sucrate was prepared from sucrose (6.697 g.; 19.56 mg.-mol.), sodium (19.56 mg.-atom) and liquid ammonia (50 ml.). Methyl iodide (1.34 ml.; 19.56 mg.-mol. + 10% excess) was then added to the reaction mixture, causing most of the precipitate to dissolve. After refluxing for 30 minutes, the ammonia was removed to give a non-reducing white solid (10.551 g.), containing sucrose only, with a strong "amine" smell.

In expt 10, a trisodium sucrate (2 g. Found: Na, 17.10; $C_{12}H_{22}O_{11}$, 80.9; NH_3 , 2.59%) was heated with methyl tosylate (2.751 g., 1 mol./sodium atom) in tri-O-methylglycerol (6 ml.) at 110° for 5 hours. After dissolving in water and deionising, evaporation gave a brown, non-reducing glass (1.165 g. Found: OMe, 11.7%. Tri-O-methylsucrose, $C_{15}H_{28}O_{11}$, requires OMe, 24.2%). Chromatography in solvent A with p-anisidine spray showed sucrose and three methylated derivatives (R_F 0.21, 0.31, 0.35).

Reaction of Methyl Iodide and Dimethylformamide on Sucrose

Sucrose (2 g.) was dissolved in dimethylformamide (25 ml.) to which methyl iodide (7.5 ml.) was added. The solution was refluxed at 75° and the rotation was measured at intervals: $[\alpha]_D + 74.1^\circ$ (initial), 41.9 (1 hr.), 44.2 (2 hr.), 49.2 (18 hr.). The final acid content of the solution was estimated by titration with 0.1 N sodium hydroxide solution using methyl red indicator and found to be 8.4 mg.-equivalents. Silver nitrate gave a heavy precipitate of silver iodide and chromatography in solvent B showed that sucrose was absent and glucose and fructose present.

Methylation of Trisodium Sucrate

Trisodium sucrate (4.223 g. Found: Na, 16.40; $C_{12}H_{22}O_{11}$, 81.3; NH_3 , 2.13%; sodium atoms/sucrose mol., 3.00) in tri-O-methylglycerol (20 ml.) was heated with methyl tosylate (5.869 g., 1 mol./sodium atom) at 110° for 5 hours with vigorous stirring under anhydrous conditions. The reaction mixture was dissolved in water (50 ml.) and deionised with Amberlite resins IR-120- H^+ and IR-45- OH^- . The solution was evaporated to a pale green glass (3.371 g. Found: OMe, 10.5%). In a second preparation the trisodium sucrate (6.082 g.) was reacted with methyl tosylate (8.457 g.) in tri-O-methylglycerol (30 ml.) to give additional methylated material (4.962 g. Found: OMe, 10.6%).

Separation of the Products of Methylation

The combined methylated products (8.33 g.) in water (10 ml.) were applied to a cellulose column (50 x 3.8 cm.)⁶¹ loosely packed by the slurry method⁵⁷, using n-butanol saturated with water as developer. Samples (1 ml.) were extracted from every fifth tube (each containing approximately 8 ml.), evaporated to dryness and analysed by paper

chromatography in solvent A with α -naphthol spray. The identification of each component found was based on the relative positions of the spots on the chromatogram, using authentic sucrose (R_F 0.10) and di-O-methylsucrose (R_F 0.33)³² as reference compounds.

From the resulting partial separation, the following fractions were selected:-

Fraction 1 (0.80 - 1.72 l.) - di-O-methylsucrose and higher methylated material (1.819 g.; 21.8%)

Fraction 2 (1.72 - 2.66 l.) - mono-O-methylsucrose and some di-O-methylsucrose (1.661 g.; 20.0%)

Fraction 3 (2.66 - 3.43 l.) - sucrose and some mono-O-methylsucrose (2.074 g.; 24.9%)

Fraction 4 (3.43 - 5.10 l.) - sucrose (1.356 g.; 16.3%)

Examination of Fraction 4

The syrup (1.35 g.) was purified by charcoal treatment and crystallisation from methanol. Crystals (0.439 g.) were obtained having $[\alpha]_D + 66.4^\circ$ (c, 2.0) and m.p. and mixed m.p. 178° , identical with authentic sucrose. (Found: C, 41.9; H, 6.47. $C_{12}H_{22}O_{11}$ requires C, 42.1; H, 6.49%). The crystalline material (0.2 g.) was acetylated by heating with sodium acetate (0.2 g.) and acetic anhydride (2 ml.) using French's method.⁶² Excess acetic anhydride was removed in a current of hot air and the resulting syrupy solid was extracted with hot benzene (10 ml.). Evaporation of the filtered benzene extract gave a syrup which crystallised from n-butanol (10 ml.). The

octa-O-acetylsucrose so prepared had m.p. 86-88°; Linstead *et al.*⁶³ reported m.p. 89° for this derivative. (Found: C, 49.2; H, 5.73. $C_{28}H_{38}O_{19}$ requires C, 49.6; H, 5.64%).

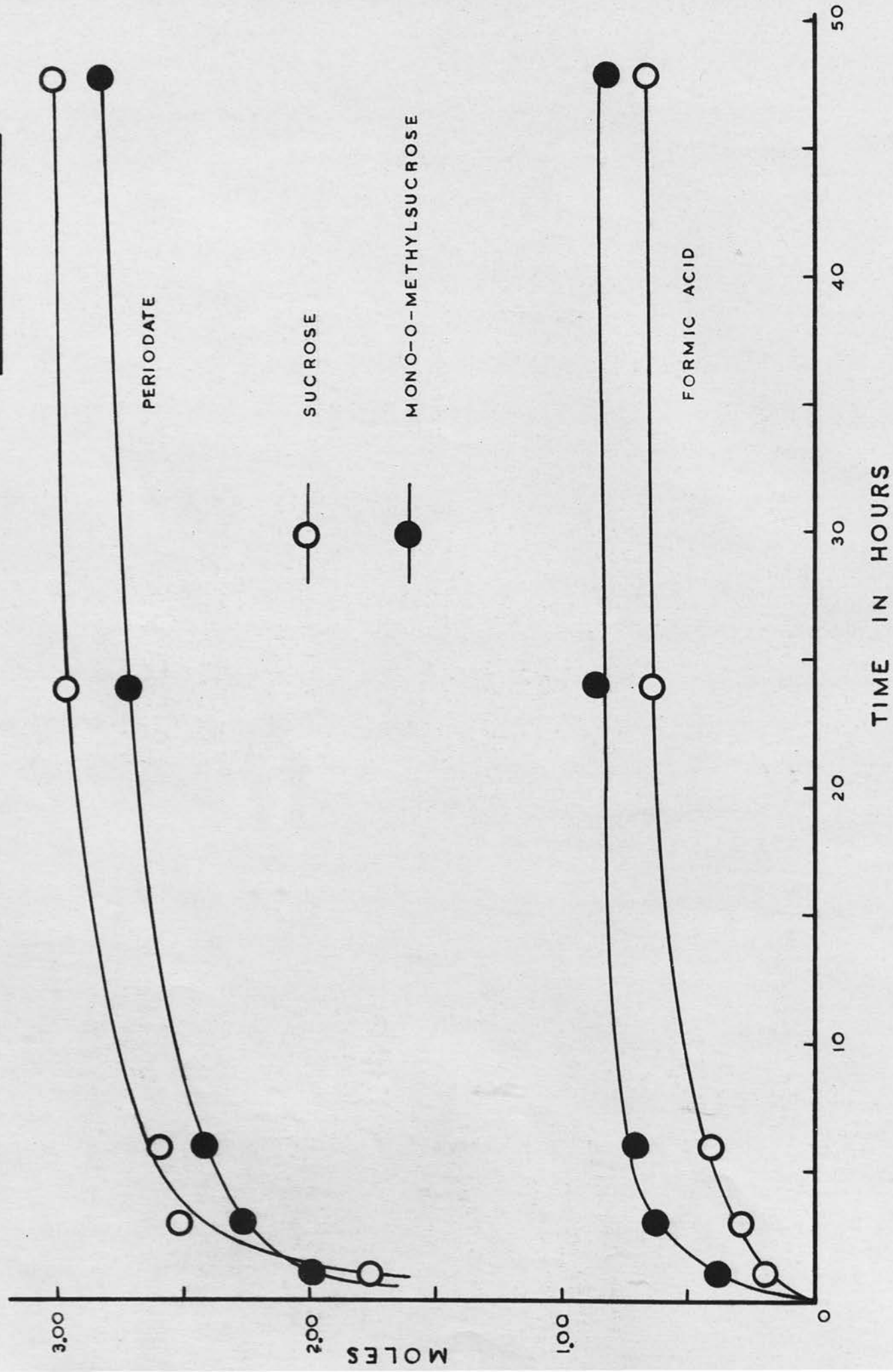
Isolation of Mono-O-methylsucrose

Fraction 2, a syrupy solid (1.66 g. Found: OMe, 11.1%) was refractionated on a tightly-packed cellulose column (55 x 3.0 cm.)⁶¹ using n-butanol saturated with water as developer. Two fractions were isolated in the manner described before, a mono-O-methyl fraction (1.35 - 2.10 l.; 0.812 g.) and a di-O-methyl fraction (0.90 - 1.31 l.; 0.241 g. Found: OMe, 17.0. Mono-O-methylsucrose, $C_{13}H_{24}O_{11}$, requires OMe, 8.7; di-O-methylsucrose, $C_{14}H_{26}O_{11}$, requires OMe, 16.8%). The mono-O-methyl fraction as a syrupy solid was purified by further refractionation on the same column, evaporation of the solvent, extraction with water (10 ml.), filtration and concentration to give a non-reducing, pale yellow glass (0.629 g. Found: OMe, 8.27%), $[\alpha]_D + 60.6^\circ$ (c, 2.1). Chromatographic analysis of the material after extended periods of development (16-48 hours) in solvent A showed only a single diffuse spot (R_{Glu} 1.11; R_{Suc} 1.44) when sprayed with α -naphthol and p-anisidine sprays. Chromatography in solvents B and C also showed a single spot. Subsequent use of solvent D (40 hr.) showed two spots (R_{Glu} 1.01, 1.15) and a trace of another (R_{Glu} 1.27).

Periodate Oxidation of Mono-O-methylsucrose

An 0.1% solution (10 ml.) of the material was allowed to react with 0.025M sodium periodate (10 ml.) in a stoppered flask in the dark at 20°; at selected intervals aliquots (2 ml.) were removed for estimation

FIGURE 2



of periodate uptake and formic acid liberated. Periodate consumption was determined by adding a measured excess of 0.1N sodium arsenite solution (1 ml.), 10% potassium iodide solution (0.5 ml.) and sodium bicarbonate (1 g.) before back-titration with 0.005N iodine solution.⁶⁴ Formic acid liberated during the reaction was estimated by direct titration with 0.002N sodium hydroxide solution, using screened methyl red indicator, after excess periodate had been destroyed with ethylene glycol (0.5 ml.).⁶⁵ Sucrose was treated similarly in a parallel experiment. Results are listed in Table 4 and plotted in Figure 2.

Table 4

Periodate Oxidation of Sucrose and Mono-O-methylsucrose

Time of oxidation (hr.)	Periodate Uptake (moles/mole of sugar)		Formic Acid Liberated (moles/mole of sugar)	
	Sucrose	Mono-O-methyl- sucrose	Sucrose	Mono-O-methyl- sucrose
1	1.76	1.99	0.19	0.38
3	2.51	2.26	0.29	0.62
6	2.59	2.41	0.40	0.70
24	2.96	2.71	0.63	0.86
48	3.01	2.82	0.65	0.81

Hydrolysis of Mono-O-methylsucrose

A portion (352 mg.) of the derivative was hydrolysed with 0.05N sulphuric acid (10 ml.) at 100° for 3 hours when $[\alpha]_D$ was found

to be -6.5° . The hydrolysate was neutralised with sodium hydroxide solution (0.1N) using phenol red indicator; ethanol (150 ml.) was then added to effect precipitation of sodium sulphate, which was removed by centrifugation. The centrifugate was evaporated to give a yellow syrup. Chromatographic analysis in solvent A revealed three spots on spraying with p-anisidine and α -naphthol: R_F 0.20, identical with glucose; R_F 0.22, identical with fructose; and R_F 0.41, corresponding to a mono-O-methylhexose.

Separation of Hydrolysate Components

The hydrolysate was applied to the tightly-packed cellulose column (55 x 3.0 cm.)⁶¹ in water (6 ml.) and developed with n-butanol saturated with water. The following fractions were obtained:-

Fraction A (0.66 - 0.91 l.) - mono-O-methylhexose fraction (135 mg.)

Found: OMe, 14.1 $C_7H_{14}O_{11}$ requires OMe, 16.0%)

Fraction B (1.20 - 1.41 l.) - fructose (58 mg.)

Fraction C (1.41 - 1.85 l.) - glucose (107 mg.)

Fraction A was shown to contain two glucose and two fructose monomethyl ethers as major components, with traces of two others, by chromatography in solvent C. Spots were readily detected as glucose or fructose methyl ethers by careful choice of sprays. E.g., α -naphthol is specific for fructose and its derivatives and gives a blue colour; p-anisidine gives a yellow colour with fructose and its derivatives and a brown colour with glucose. Glucose and fructose, and their methyl ethers, can also be identified, although less readily, with

benzidine. The components of this syrup (122 mg.) were then separated by quantitative thick paper chromatography⁶⁶ using Whatman 3 mm. paper, previously refluxed overnight in benzene/ethanol (1:1). The material was applied in aqueous solution (1 ml.) to two papers (28" x 12") which were developed with solvent C for 64 hours. After side-strips had been sprayed with benzidine and α -naphthol, portions were cut out and eluted alternately with water (5 x 10 ml.) and ethanol (5 x 10 ml.). The eluates were concentrated to give yellow syrups which were purified by charcoal treatment in ethanolic solution; colourless syrups were obtained on removal of the ethanol. The following separation of glucose and fructose monomethyl ethers was thus effected:

<u>Mono-O-methylglucoses</u>	<u>Mono-O-methylfructoses</u>
Component A1 : 13.1 mg.; 10.7% of fraction A	Component A4 : 27.7 mg.; 22.8% of fraction A
" A2 : 31.8 mg.; 26.1% of fraction A	" A5 : 19.2 mg.; 15.7% of fraction A
" A3 : 5.6 mg.; 4.6% of fraction A	" A6 : 7.1 mg.; 5.8% of fraction A

The four major components A1, A2, A4 and A5 were compared chromatographically with authentic monomethyl ethers of glucose and fructose in solvent C with a development time of 2-3 days using α -naphthol, p-anisidine and benzidine sprays, when the following results were obtained:-

Table 5

R_{Glu} and R_{Fru} Values of the Mono-O-methylhexose Components

Glucose monomethyl ethers	R _{Glu}	Fructose monomethyl ethers	R _{Fru}
2-O-methyl-D-glucose	4.83	1-O-methyl-D-fructose	3.69
3-O-methyl-D-glucose	5.25	3-O-methyl-D-fructose	4.06
4-O-methyl-D-glucose	4.64	4-O-methyl-D-fructose	4.04
6-O-methyl-D-glucose	4.08	A4	3.62
A1	4.03	A5	4.02
A2	5.00		

It was observed that fractions A2 and A5 partially crystallised in the form of prismatic needles and small platelets respectively but not in sufficient quantity to give m.p. determinations.

Preparation of 1,2:4,5-Di-O-isopropylidene-D-fructose from Fraction B

The colourless syrup (58 mg.) was shaken for 5 hours with anhydrous acetone (5 ml.) containing a trace of sulphuric acid (1 drop/10 ml. acetone).⁶⁷ Water (10 ml.) was added to the colourless solution which was neutralised with sodium carbonate. Acetone was then removed at 30° under reduced pressure. The residual aqueous solution was extracted with chloroform (3 x 20 ml.) and the combined extracts were dried overnight over anhydrous sodium sulphate before the solvent was removed. The product (33.7 mg.) was obtained as long fine needles by crystallisation from petrol-ether (b.p. 60-80°; 5 ml.), m.p. and mixed m.p. 117-8°, $[\alpha]_D^{23} - 143^\circ$ in chloroform (c, 0.95). In a parallel experiment conducted

under the same conditions, 1,2:4,5-di-O-isopropylidene-D-fructose (23.4 mg.) was obtained from D-fructose (40 mg.), m.p. $117-8^{\circ}$, $[\alpha]_D^{20} - 145^{\circ}$ in chloroform (c, 0.835). Bell⁶⁷ quotes m.p. 119° and $[\alpha]_D - 147.3^{\circ}$ in chloroform for this derivative.

Preparation of Penta-O-acetyl- β -D-glucose from Fraction C

The pale brown syrup (107 mg.) was heated at 100° with anhydrous sodium acetate (50 mg.) and acetic anhydride (1 ml.) for 2 hours with occasional shaking. The addition of ice-cold water (10 ml.) caused the formation of a heavy oil which solidified on further cooling. The resultant white solid was triturated with ice-cold water and dried in vacuo over phosphorus pentoxide. The white powder (115 mg.) was recrystallised firstly from aqueous ethanol and then absolute ethanol to give colourless needles (62 mg.), m.p. and mixed m.p. 128° , $[\alpha]_D^{22} + 4.5^{\circ}$ in chloroform (c, 4.9). In a control experiment a quantity of crude acetate (137 mg.) with identical properties to the above was obtained from D-glucose (100 mg.).

Hudson and Dale⁶⁸ quote m.p. 132° , $[\alpha]_D^{20} + 3.8^{\circ}$ in chloroform for this derivative.

Periodate Oxidation of Mono-O-methylhexose Components

The method of quantitative microestimation of formaldehyde was that of Hough, Powell and Woods.⁴⁷ D-Mannitol was used as a source of standard amounts of formaldehyde for the construction of a calibration graph, periodate oxidation in the presence of sodium hydrogen carbonate buffer giving 2.0 mol. of formaldehyde.⁴⁷

Portions (1, 2, 5 and 10 ml.) of an 0.015% mannitol solution together with 0.25M sodium periodate solution (2 ml.) and solid sodium hydrogen

Table 6

Periodate Oxidation of Mono-O-methylhexose Components

Sugar	Time of oxidation at 25°											
	20 min.			3 hr.			24 hr.			48 hr.		
	P.U.	HCHO	F.A.	P.U.	HCHO	F.A.	P.U.	HCHO	F.A.	P.U.	HCHO	F.A.
Component A1	1.9	0.14		2.5	0.15		2.4	0.15	1.6	2.5	0.14	1.8
Component A2	3.0	0.09		3.8	0.10		3.9	0.28	1.5	4.5	0.36	2.0
2-O-methyl-D-glucose	3.9	0.13	0.60	4.4	0.17	1.3	4.5	0.43	1.9	4.8	0.58	
Component A4				3.1	0.18		3.2	0.33	2.1	3.2	0.52	2.2
1-O-methyl-D-fructose				3.5	0.28		3.7	0.74	2.5	3.6	0.75	2.6
Component A5				0.5	0.25		0.6		0.2	0.8	0.30	0.3
3-O-methyl-D-fructose				3.1	0.98		3.4	0.98	1.8	3.4	0.98	1.6
4-O-methyl-D-fructose				1.4	1.02		1.8	1.04		1.8	1.16	0.7
										2.7	1.18	0.8

P.U. = moles of periodate consumed/mole of sugar

HCHO = moles of formaldehyde produced/mole of sugar

F.A. = moles of formic acid produced/mole of sugar

carbonate (0.5 g.) were made up in each case to a standard volume (20 ml.). After oxidation in the dark at 20° for 90 minutes, which had been found to give maximum formaldehyde production, aliquots (2 ml.) were removed and excess periodate destroyed by precipitation with saturated barium chloride solution (2 ml.). After centrifuging, a portion (2 ml.) of the supernatant was mixed with phenylhydrazine hydrochloride reagent (2 ml.) and the combined solution left for 30 minutes. Acidified potassium ferricyanide reagent (7 ml.) was then added and after 3 minutes the solution was diluted to 50 ml. Absorptions of the red-coloured solutions were compared in 4 cm. cells at 518 $m\mu$ against a blank determination prepared similarly, using a "Unicam" SP.600 spectrophotometer:-

Optical density	0.100	0.170	0.463	0.893
Formaldehyde (μ g.)	2.48	4.95	12.38	24.76

Periodate oxidations of the four mono-O-methylhexose components were carried out in unbuffered solution (20 ml.) containing 0.25M sodium periodate solution (2 ml.) at 20° in the dark and compared with authentic samples in the same manner. Aliquots were removed at selected intervals for estimation of formaldehyde (2 ml.) as above and for periodate uptake (2 ml.) and formic acid production (1 ml.) as previously described. Results are summarised in Table 6.

Identification and Characterisation of Mono-O-methylhexose Components

Syrup A1 (13.1 mg. Found: OMe, 14.9. $C_7H_{14}O_6$ requires OMe, 16.0%) was tentatively identified by chromatography as 6-O-methyl-D-glucose (Table 5), $[\alpha]_D + 53^\circ$ (c, 0.5). $[\alpha]_D + 59, 55^\circ$ is reported for 6-O-methyl-D-glucose.⁶⁹

Periodate oxidation of this component was carried out, aliquots being removed at 20 minutes, 3, 24 and 48 hours. Confirmation of these results was obtained by the partial periodate oxidation method of Lemieux and Bauer⁴⁸ in which quantities (1 mg.) of the sugars were oxidised with periodate (0.5N NaIO₄, 0.12 ml.) at 0° for 1 hour, when excess periodate was destroyed with ethylene glycol (2-3 mg.). The formyl esters so formed were saponified with sodium hydroxide solution (0.5N) before chromatography of the solutions in solvent A with aniline phthalate spray.

Component A1 and 6-O-methyl-D-glucose gave identical yellow spots, R_F 0.78, with fainter spots, R_F 0.60.

The phenylosazone of this component was prepared by Smith's method.⁷⁰ Syrup A1 (9.4 mg.) 10% (v/v) acetic acid (0.5 ml.) and freshly distilled phenylhydrazine (0.04 ml.) were heated at 80° for 3 hours, which precipitated 6-O-methyl-D-glucose phenylosazone (2.9 mg.). After centrifuging, washing with ethanol and rapid drying, the derivative had m.p. 184° (literature⁶⁹, 184-7°). Found: OMe, 7.7.

C₁₉H₂₄O₄N₄ requires OMe, 8.3%.

Syrup A2 (31.8 mg. Found: OMe, 15.5%), probably 2-O-methyl-D-glucose by chromatography, (Table 5), had $[\alpha]_D + 58^\circ$ (c, 1.5). Literature⁶⁹ quotes $[\alpha]_D + 66^\circ$ for 2-O-methyl-D-glucose.

Periodate oxidation was carried out as before for A1 and the results (Table 6) were again confirmed by the partial periodate oxidation method⁴⁸, component A2 and 2-O-methyl-D-glucose both giving strong, lemon-yellow spots, R_F 0.28, in the cold.

Testing with freshly prepared Wallenfel's spray (triphenyl-tetrazolium hydroxide)⁴⁹ did not produce a bright-red formazan compound

with either A2 or 2-O-methyl-D-glucose, indicating that position 2 was substituted.⁵⁰

When treated similarly to A1 above, syrup A2 (24.8 mg.) gave D-glucose phenylosazone, m.p. 205° after ethanol washing, with negligible methoxyl content.

Syrup A4 (27.7 mg. Found: OMe, 15.1%), which appeared to be 1-O-methyl-D-fructose by chromatography (Table 5) had $[\alpha]_D - 64.5^\circ$ (c, 1.1). Literature⁷¹ quotes $[\alpha]_D - 82.3^\circ$ at equilibrium for crystalline 1-O-methyl-D-fructose.

Periodate oxidations of this component and an authentic sample of 1-O-methyl-D-fructose were carried out as before, aliquots being removed over 3-72 hours, and results are recorded in Table 6.

The remainder of A4 (12.1 mg.) was treated with phenylhydrazine in acetic acid solution as before to give D-glucose phenylosazone (4.4 mg.), m.p. 208° (after washing with ethanol) and mixed m.p. 207°, with negligible methoxyl content.

Syrup A5 (19.2 mg. Found: OMe, 15.8%), either 3- or 4-O-methyl-D-fructose by chromatography (Table 5), had $[\alpha]_D - 55^\circ$ (c, 1.8). Haworth et al.⁵¹ have reported $[\alpha]_D - 53.5^\circ$ at equilibrium for crystalline 3-O-methyl-D-fructose.

Periodate oxidation of this syrup, after drying over phosphorus pentoxide at 55°/0.05 mm., was compared with that of authentic 3- and 4-O-methyl-D-fructose (Table 6) and ionophoresis of this syrup showed a major component having M_G 0.0. Found: 1-O-methyl-D-fructose, M_G 0.69; 4-O-methyl-D-fructose, M_G 0.64.

Attempts to form the phenylosazone of this component were unsuccessful.

SUMMARY and CONCLUSIONS

Investigations into the preparation of sodium sucrates using conventional solvents such as morpholine and pyridine as reaction media were unsuccessful. Various derivatives were isolated but these analysed more closely to addition compounds of sucrose, $C_{12}H_{22}O_{11} \cdot xNaOH$, containing solvent residue, rather than substitution products, $C_{12}H_{22-x}O_{11}Na_x$.

Liquid ammonia was employed successfully to prepare true sodium sucrates, which were isolated with as much as one mole of ammonia (4.47%) in the case of the crystalline monosodium sucrate, $C_{12}H_{21}O_{11}Na \cdot NH_3$. Higher substituted sucates contained much less ammonia (1.4 - 2.7%) which nevertheless still interfered considerably with subsequent methylation, due to preferential reaction of the methylating reagent with the ammonia and the release of free acid. With highly substituted sucates this side-reaction caused a 'stripping' of a proportion of the sodium atoms, but with monosodium sucate methylation was prevented completely. Consequently, monosodium sucate could not be methylated with methyl iodide using liquid ammonia, acetone, dimethylformamide or dimethylsulphoxide as solvent media.

The failure of methyl iodide to condense satisfactorily with sodium sucates in dimethylformamide was due to interaction between the methylating agent and dimethylformamide with the liberation of free acid.

Trisodium sucate was methylated with methyl tosylate in the presence of tri-O-methylglycerol to give a mixture of sucrose and sucrose methyl ethers. Little previous use appears to have been made

of this powerful methylating agent in carbohydrate chemistry.⁷⁶

A mono-O-methylsucrose was isolated from the mixture by cellulose column chromatography and appeared initially to be homogeneous. Periodate oxidation followed closely that of sucrose, indicating that substitution on secondary hydroxyl groups to any great extent was excluded, a result which could not be confirmed by hydrolysis experiments.

Hydrolysis of the mono-O-methylsucrose produced a mixture of sugars which were separated by cellulose column chromatography into D-fructose (16.5%), D-glucose (30.4%) and a mono-O-methylhexose fraction (38.4%). The latter was separated by thick paper chromatography and the following sugars were characterised:-

2-O-methyl-D-glucose (2.4 parts), 6-O-methyl-D-glucose (1.0 part), 1-O-methyl-D-fructose (2.1 parts) and probably 3-O-methyl-D-fructose (1.5 parts). Two other unidentified mono-O-methylhexoses were present in smaller amounts.

From the relative proportions of unsubstituted glucose and fructose present, substitution would appear to have occurred to a greater extent on the fructose moiety than on the glucose.

The distribution of mono-O-methylhexoses indicates that substitution occurs mainly on C_2 and, to a lesser extent, on C_6 of the glucose; in the fructose portion C_1 is substituted rather more than C_3 . These results agree with previous views on the relative reactivities of hydroxyl groups in glucose and in glucose polysaccharides, mainly cellulose.⁷² In alkaline media substitution would seem to favour C_2 rather more than C_3 or C_6 , although it must

be remembered that the latter position is frequently masked by hydrogen bonding in the case of cellulose. Rogovin et al.⁷³ have shown that in methylation of cellulose with sodium isoamyloxide and methyl iodide, the number of methyl groups formed on secondary hydroxyl groups greatly exceeded that on primary hydroxyl groups. More recently the rate constants have been measured for the methylation of alkali cellulose with methyl chloride and shown to be in the ratio 5:1:2 for C₂:C₃:C₆ of each glucose unit.⁷⁴

It has been claimed⁷⁵ that, in sucrose heated with n-butanol and caustic soda, the first atom is associated with C₂ of the glucose, the second with C₃ of the fructose and the third with C₃ of the glucose.

To conclude, it must be borne in mind that over half of the sodium atoms originally present in the sucrate were not replaced by methyl groups, having been removed prior to methylation by the acid products of the ammonia side-reaction. It is debatable, therefore, whether the location of the methyl groups in the mono-O-methylsucrose is fully representative of the positions of the sodium atoms in trisodium sucrate. Present work indicates that, to substantiate this relationship, further work would be necessary on the composition of higher methyl ethers of sucrose prepared by this method and that it would be an extremely complex problem; moreover, the presence of ammonia makes it impossible to locate all the sodium atoms originally present.

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